

b. enzymatically reducing said oxidation product to 2-KLG wherein said enzymatic reduction requires a reduced form of said enzymatic co-factor,

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between and coupled to the first oxidizing step and the reducing step wherein the oxidized form of said co-factor is selected from the group consisting of NADP⁺, NAD⁺, ATP, ADP, FAD and FMN,

wherein said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, and 6-carbon sugar acids, and is capable of being converted to an ascorbic acid (ASA) intermediate.

C1
cont
18. (Twice amended) A process for the non-fermentative production of 2-KLG from a carbon source, comprising the following steps in any order:

a. enzymatically oxidizing the carbon source by a first oxidative enzymatic activity to a first oxidation product;

b. enzymatically oxidizing the first oxidation product by a second oxidative enzymatic activity to a second oxidation product;

c. enzymatically oxidizing the second oxidation product by a third oxidative enzymatic activity to a third oxidation product; and

d. enzymatically reducing the third oxidation product by a reductase enzyme to 2-KLG

wherein at least one of said first, second and third oxidative enzymatic activities requires an oxidized form of an enzymatic co-factor and said reductase enzyme requires a reduced form of said enzymatic co-factor, wherein the oxidized form and the reduced form of said co-factor are recycled between and coupled to at least one oxidizing step and the reducing step

wherein the oxidized form of said co-factor is selected from the group consisting of NADP⁺, NAD⁺, ATP, ADP, FAD and FMN, and

wherein said carbon source is selected from the group consisting of 6-carbon sugars, mixtures of 6-carbon sugars, 6-carbon sugar acids, and is capable of being converted to an ascorbic acid (ASA) intermediate.

C2
20. (Once amended) The process of Claim 18 wherein said first oxidative enzymatic activity requires an oxidized form of said enzymatic co-factor.

C4
25.(Once amended) ~~The process of Claim 18 wherein at least one of said first, said second, said third and said fourth enzymatic activities are immobilized.~~

C4
26.(Once amended) ~~The process of Claim 18 wherein at least one of said first, said second, said third and said fourth enzymatic activities are in solution.~~

C5
31.(Once amended) ~~The process of Claim 29 wherein said reductase activity is obtainable from *Corynebacterium* or *Erwinia*.~~

47.(Twice amended) The process of Claim 15 or Claim 18 that is continuous.

48.(Twice amended) The process of Claim 15 or Claim 18 that is batch.

C6
49.(Twice amended) The process of Claim 15 or Claim 18 that proceeds in an environment comprising organic solvents.

C6
50.(Twice amended) The process of Claim 15 or Claim 18 that proceeds in an environment comprising long polymers.

51.(Twice amended) The process of Claim 15 or Claim 18 further comprising the step of obtaining ASA from said 2-KLG.

63.(Once amended) A process for the non-fermentative production of 2-KLG from glucose comprising the following steps:

- a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;
- b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;
- c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and
- d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

C7
wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between and coupled to the glucose oxidizing step and the reducing step and wherein the oxidized form of said co-factor is NADP⁺ or NAD⁺.

73.(Once amended) A process for the non-fermentative production of 2-KLG from glucose comprising the following steps:

- a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;
- b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;
- c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and
- d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between and coupled to the glucose oxidizing step and the reducing step, and wherein the oxidized form of said co-factor is NADP⁺ or NAD⁺,

wherein the process proceeds in an environment wherein the 2,5-DKG reductase is provided exogenously to a host cell.

77.(Once amended) The process of Claim 80 wherein the host cells are viable.

78.(Once amended) The process of Claim 80 wherein the host cell is non-viable.

79.(Once amended) The process of Claim 80 wherein the host cell is modified to eliminate the naturally occurring GDH activity and a heterologous GDH having a specificity for NADP⁺ or NAD⁺ is introduced into said host cell.

Please add the following new claims.

80. A process for the non-fermentative production of 2-KLG in an environment comprising host cells, comprising the following steps in any order,

- a. enzymatically oxidizing glucose by a glucose dehydrogenase to produce a first oxidation product, wherein said oxidation requires an oxidized form of an enzymatic co-factor;
- b. enzymatically oxidizing said first oxidation product to produce a second oxidation product;
- c. enzymatically oxidizing said second oxidation product to produce a third oxidation product; and

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d. enzymatically reducing said third oxidation product to 2-KLG, wherein said reduction requires a reduced form of said enzymatic co-factor

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between and coupled to the first oxidizing step and the reducing step and said oxidized co-factor is NAD⁺ or NADP⁺ and said reduced co-factor is NADH or NADPH.

C 10
Cnt

81. The process of Claim 80 wherein the oxidized co-factor is NAD⁺ and the reduced co-factor is NADH.

82. The process of Claim 80 wherein the oxidized co-factor is NADP⁺ and the reduced co-factor is NADPH.

Add 24